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USING THE METHOD OF SEPARATION AND IDENTIFICATION OF AMINO ACIDS TO DETECT EXTRATERRESTRIAL LIFE

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USING THE METHOD OF SEPARATION AND IDENTIFICATION OF AMINO ACIDS TO DETECT EXTRATERRESTRIAL LIFE

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ABSTRACT. A method is developed for separating amino acids from soils on the Earth and for analyzing them. The development of an automatic system which would allow amino acids in soil samples from other planets, to be analyzed by automatic space stations, ir proposed.

Space research has reached the stage where it is possible to study /3* organic material in other planets with the aid of automatic stations. The greatest interest is primarily in biologically-important compounds such as proteins, nucleic acids and their derivatives. Their discovery on other planets would indicate the possible existence of certain forms of life there.

In searching for these compounds, exobiology could use the advancements of biogeochemistry, which is occupied with studying organic biologically-important compounds in Earth soils and sediments. The experience accumulated by biogeochemisty and methods it has developed have already been used successfully in analyzing organic material from meteorites [15, 20, etc.] and lunar samples in the Apollo program [11, 12, 21].

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*Numbers in the margin indicate pagination in the original foreign text.

Of the many methods of analyzing soil samples, only a few do not require preliminary treatment of the samples (microbiological methods, pyrolysis with subsequent analysis on the gas chromatograph, and several others). The majority of these analytical methods demand certain preparations be made before analysis. This relates especially to analyzing biologically-important compounds, particularly, to amino acids, which we shall discuss later.

To analyze soil samples from other planets with the aid of spacecraft, it is necessary to create special automatic control systems which prepare the samples for analysis and perform the analyzing itself according to a set program. Therefore, the aim of this work is to develop a method for separating amino acids from Earth objects and subsequently analyzing them, aimed at objects depleted of organic matter. On the basis of this method, the creation of an automatic analysis system is suggested. /4

As is known, there are comparatively few free amino acids in the soil. They are mainly found in a bound state: either they form groups with humic acids [4, 5, 7, 9, 14, 19, 24, 25, 26, 27, 34, 37], or are members of mineral-organic complexes [1, 2, 3, 22 etc.], or are found in intracellular material of soil micro-organisms. As a result, many difficulties arise in extracting amino acids from soils. Even the best methods of extracting amino acids do not extract more than 1% of the amount of amino acids produced after hydrolysis [28]. Therefore, acid hydrolysis is used for maximum extraction of amino acids from soils [6 н HCl , 105° C, 24 hours]. But even in this case, extraction of amino acids is not always complete [27, 32, 33] because of the formation of strong bonds between the amino acids and polyvalent metals, which are components of mineral clays. These bonds are broken down by preliminary processing of the soil, by diluted fluoric acid [31, 32, 33 etc.] or by the use of subsequent acid or alkali hydrolysis [27].

The study of organic nitrogenous soil compounds, particularly amino acids, was the subject of a survey recently published by Bremner [6]. From the latest works on analyzing amino acids in soils and sediments, we must note /5

works [7, 10, 16, 17, 18, 28, 29, 30, 31, 32, 33, 35, 36, 39], analyzed in detail in the recent survey [38].

The objects of research in developing a method of separating amino acids from soils were: black earth from the Kiev region; soil, depleted of organic matter — green-blue barren soil from the Kapet-Dag mountains — and river sand, fired at 900° C for five hours. The microbiological population of the barren soil was $\sim 5 \cdot 10^5$ per gram of air-dry soil; for black earth it was approximately two orders of magnitude more.

Used in the work were core columns I (18 x 3 cm) and II (7 x 2 cm) with cation-exchange resin Dowex 50w x 4 (100 - 200 mesh) in hydrogen form and anion-exchange resin Dowex I (200 - 400 mesh) in OH^- form, respectively. Dowex resin 50w x 4 was twice preliminarily washed with a 2N solution of NaOH, with water, and with a 4N solution of hydrochloric acid, after which it was equilibrated by acidified water, pH 1.5. Dowex resin I was washed twice with 1N solution of hydrochloric acid, water, 1N solution of NaOH, and then it was equilibrated with a 2N solution of NH_4OH.

Ten grams of soil, crushed to a powder in a porcelain mortar, were flooded with 50 ml of 8N hydrochloric acid. To prevent destruction of the amino acids from the effect of atmospheric oxygen, a bulb with the contents was pumped out by a water jet pump. Hydrolysis of the soil was then carried out at 105 - 110° C for 24 hours.

After the hydrolysate was filtered through a glass filter G - 3 /6
under pressure, the residue in the filter was washed with 8N HCl until the yellow color disappeared in the filtrate. Then, the hydrolysate was steamed dry on a rotary evaporator at 50 - 60° C. The residue was dissolved in distilled water, the solution reduced to pH 1.5, filtered through a glass filter G - 4 and fed to column I. After the filtered solution passed through the column, the resin was washed by distilled water until extinction of $\varepsilon/\lambda = 275 m\mu$ was not less than 0.02 - 0.03 of the optical unit. The

elution was conducted by a 2N solution of NH_4OH, which desorbs amino acids, but inorganic cations (Na^+, Ca^{++}, Mg^{++}, etc.) remained in the resin. The rate of flow through the column during sorption and elution was 36 ml/hr per cm^2; in washing with water, it was 60 ml/hr per cm^2.

Sampling of fractions took place in a collector of the Ultzozac ZKB company with 5 - 10 ml. Only fractions with alkali pH gave a positive reaction to ninhydrin. These fractions were collected together (eluate 1), and fed into column II. After eluate 1 passed through the column, it was washed with a 2N solution of NH_4OH to remove unsorbed material (to $E^{(1)} < 0.02$ at discharge from the column with $\lambda = 275$ mm). Then the column was washed with a small amount of water to remove NH_4^+ ions. The elution was conducted by a 0.1 N solution of HCl. The rate of flow in sorption, washing, and elution was 90 ml/hr, 180 ml/hr, and 36 ml/hr, respectively. Fractions were removed by 5 ml in sorption and washing, and by 2 ml in elution. Fractions of the eluate giving a positive reaction to ninhydrin were collected together, evaporated /7 dry on a rotary evaporator at 40° C and were analyzed in the "Unichrom" amino acid analyzer of the Backman company. Several fractions during the passage also had a positive ninhydrin reaction.

The ninhydrin reaction was carried out by Moore and Stein [23].

In isolating amino acids from green-blue barren soil deprived of organic matter, a positive ninhydrin reaction could only be noted by concentrating the fractions to small volumes. Fractions with pH > 7.5 - 8.0 (column 1) were concentrated, as a ninhydrin — positive reaction was observed only in fractions with alkali pH. In the case of column II, quite a large volume of the eluate was concentrated to provide as much discharge as possible from the column of ninhydrin positive compounds.

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(1) E — extinction coefficient.

Regarding the fired river sand, a ninhydrin-positive reaction was not successfully detected on the paper even with concentration, and selection of fractions in this case was carried out analogously with previous experiments (black earth and green-blue barren soil).

Of the existing methods of desalinizing solutions (gel-filtration, dialysis, extraction, use of polyacrylamide gels, etc.) a method was chosen in which the desalinization process is conducted in an ion-exchange resin, in particular, in a Dowex resin 50 w x 4 (H^+). This method is effective, simple, it does not demand heavy weights, which is especially important in space studies, and most important — it allows desalinization to be combined with partial purification of the amino acids from impurities.

As is known, amino acids can carry positive as well as negative charges /8 depending on pH. This is their nature, and it was used in this work in cleansing the amino acids of other components of the soil hydrolysate. With acidic pH (1.5 - 2.0) all the carboxyl groups of amino acids are nondissociated ($-COO^- + H^+ \rightarrow -COOH$; $pH < pK_1$, where $K_1 = \frac{[COO^-] \cdot [H^+]}{[COOH]}$ — first constant of dissociation, $[COO^-]$, $[H^+]$, [COOH] are concentrations; pK_1 of amino acids lies within the limits 3.5 - 5.5), whereas amino groups are charged ($NH_2 + H^+ \rightarrow NH_3^+$ $pH < pK_2$, where $K_2 = \frac{[NH_2] \cdot [H^+]}{[NH_3^+]}$ — second constant of dissociation, $[NH_2]$, $[H^+]$, $[NH_3^+]$ are concentrations; pK_2 of amino acids lies within limits 7 - 9). Therefore, all amino acids are cations and are sorbed in cation-exchange resin Dowex 50 w (H^+), exchanging with ions of hydrogen. All neutral and negatively charged matter of the hydrolysate passes through the column, not being sorbed in the resin, and leaves the column when it is cleansed with water. For alkali pH (> 11.0), all amino acids are charged negatively, as amino groups are not charged ($pH > pK_2$). Therefore, in Dowex anion-exchange resin 1 at pH 11.7 all amino acids are sorbed, whereas cations and neutral molecules are washed out of the column. In particular, amino sugars (glucosamine and galactosamine), present in the soil, pass through the column. This is explained by the positive ninhydrin

reaction observed in passing through column II. Thus, it is possible to a considerable degree to purify amino acids of impurities which interfere with further analysis.

Data from amino acid analysis of black earth, green-blue barren soil and river sand are given in the table (in micromoles per 1 gram of sample). /9

Bsic amino acids were not analyzed in river sand. The absence of quantitative characteristics for tyrosine and phenylalanine in the case of black earth is due to the small content of these amino acids in the hydrolysate in comparison with other amino acids. In converting micromoles to milligrams, we find that the amount of amino acids in black earth, barren soil and fired river sand are approximately 1, $2 \cdot 10^{-2}$, and $4 \cdot 10^{-4}$ mg per gram of the sample, respectively. /10

As can be seen from the table, the content of glycine and alanine in the samples studied is much higher than other amino acids (in barren soil it is 18% and 24%, respectively; in black earth, about 22%). It is possible that part of the glycine and alanine are products of the disintegration of other amino acids, both in the soil and in the separation process [13]. In all the experiments conducted, arginine and tryptophan were absent. Protein amino acids (citrulline, ornithine, etc.) whose presence in many soils and sediments is now considered definitely established [6, 8, 13], were not detected.

Reproducibility of the results of amino acid analysis from test to test was good (variations in % of amino acid content usually did not exceed several percent).

If, in working with black earth, we found that the impurities introduced into the samples at various stages of their processing were not significant because of the high content of soil organic matter, then in turning to green-blue barren soil, depleted of organic matter, it was necessary to make controlled experiments to evaluate possible impurities of the samples in the

Amino acids	Black earth	Green-blue barren soil	River sand, fired at 900° C for five hours
Aspartic acid	0,070	0,006	0,0002
Trionine	0,708	0,012	0,0002
Serine	0,800	0,017	0,0008
Glutamic acid	0,076	0,004	0,0002
Proline	1,268	0,017	traces
Glycine	2,254	0,036	0,0010
Alanine	2,200	0,047	0,0004
Valine	1,176	0,017	0,0002
Isoleucine	0,596	0,010	0,0001
Leucine	0,640	0,020	0,0002
Tyrosine	traces	0,001	--
Phenylalanine	traces	0,006	traces
Lysine	0,296	0,012	
Histidine	0,054	0,001	
Total amount of amino acids	10,138	0,206	0,0035

process. For this, river sand, fired at 900° C for five hours was also used. Amino acid analysis of river sand shows that the amount of amino acid in the hydrolysate of the latter is only ∿ 1.5% of the amount obtained from green- /11
blue barren soil — that is, approximately $3 \cdot 10^{-9}$ M per gram of sample, or on the order of 10^{-10} M of each amino acid per gram of the sample. This roughly agrees with data [10^{-10} - 10^{-11} M] in evaluating possible impurities in studying the amino acid content of samples of lunar soil [11, 12, 21]. This evidently indicates that amino acids, detected in green-blue barren soil, are not products of contamination.

As green-blue barren soil contains extremely little organic matter, and their microbiological population is nevertheless quite high (∿ 10^5 per gram of air-dry soil), it can then be assumed that the main part of separated amino acids is originally contained in intracellular matter of microorganisms.

The level of sensitivity of amino acid analysis in this work is ∿ 10^{-9} M. However, to analyze amino acids in space research, such sensitivity is hardly adequate [11, 12, 21]. Therefore, for amino acid analysis of samples studied in this work, we used the method of converting the amino acids to volatile compounds with subsequent analysis in a gas chromatograph (sensitivity of the method, 10^{-11} M) and adequate results were obtained. Despite the purely qualitative character of the analysis conducted, it can be stated confidently that the technique of separating and purifying amino acids of impurities, described in this work, is completely acceptable for subsequent esterification and chromatographic analysis.

Thus, in this work a method has been worked out for separating amino acids from soils, concentrating them, and partially cleansing them of impuri- /12
ties. This technique makes it possible to analyze amino acids of samples in a gas chromatograph with preliminary esterification of the separated amino acids. On the basis of this technique and the methods of analyzing amino acids in a gas chromatograph, it is possible to create an automatic system which

would allow amino acids in soil samples from other planets to be analyzed with the aid of automatic space stations.

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